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(21) International Application Number: PCT/US99/16698 (22) International Filing Date: 5 August 1999 (05.08.99) (30) Priority Data: 09/129,988 5 August 1998 (05.08.98) US (71) Applicant: THERMOGENESIS CORP. [US/US]; 3146 Gold Camp Drive, Rancho Cordova, CA 95670 (US). (72) Inventors; and (73) Inventors/Applicants (for US only): COELHO, Philip, Henry [US/US]; 121 Giotto Way, El Dorado Hills, CA 95762 (US). GODSEY, James, H. [US/US]; 101 Summer Shade Court, Folsom, CA 95630 (US). BRAUSCH, Jim [US/US]; 6875 Villa Juarez Circle, Sacramento, CA 95828 (US). KINGS- LEY, Phil [US/US]; 9560 Appalachian Drive, Sacramento, CA 95827 (US). ROCK, Gail [CA/CA]; 270 Sandridge Road, Ottawa, Ontario K1L 5A2 (CA). (74) Agent: KRETEN, Bernhard; Suite 245, 77 Cadillac Drive, Sacramento, CA 95825 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: APPARATUS AND METHOD OF PREPARATION OF STABLE, LONG TERM THROMBIN FROM PLASMA AND THROMBIN FORMED THEREBY		
(57) Abstract A sterile method for preparing stable thrombin component from a single donor's plasma in which the thrombin component is harvested simultaneously from the clotting and adhesive proteins component from the same donor plasma in less than one hour. The combined components provide an improved biological hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The thrombin provides polymerization of the clotting and adhesive proteins in less than five seconds, and is sufficiently stable to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by diluting the thrombin with saline.		

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Apparatus and Method of Preparation of Stable, Long Term Thrombin from Plasma and Thrombin Formed Thereby

Technical Field

5 The following invention relates generally to the preparation of thrombin enzyme from a given unit of plasma, which is sufficiently stable that it provides rapid clotting of a fibrinogen-rich solution of clotting and adhesive proteins for more than six hours.

Background Art

10 Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

15 Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that
20 CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the perioperative theater with the patient close by, thereby avoiding the need to initiate the process days in advance.

 These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical
25 hemostasis and tissue adhesion. Commercially available thrombin, however, is generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the CryoSeal™ cryoprecipitation invention is that the
30 harvested clotting and adhesive proteins sourced from the patient's own blood eliminates the risk of contamination by infectious blood-borne disease when these

clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

For instance, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994) clearly describes an improved fibrin glue in which the thrombin component whose preparation method, - adjusting the ionic strength of the blood and pH of the plasma to cause precipitation of a thrombin component for later resolubilization - was described therein, would be combined with a fibrinogen component also sourced from the plasma of the same donor. These steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192) follows the lead of Cederholm-Williams by also teaching a method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which the fibrinogen in the plasma is first precipitated to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute

window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

- 5 The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the
 10 nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

U.S. PATENT DOCUMENTS

<u>PATENT NO.</u>	<u>ISSUE DATE</u>	<u>INVENTOR</u>
5,648,265	July 15, 1997	Epstein
5,510,102	April 4, 1996	Cochrum
15 5,585,007	December 17, 1996	Antanavich, et al.
5,605,887	February 25, 1997	Pines, et al.
5,614,204	March 25, 1997	Cochrum
5,631,019	May 20, 1997	Marx
5,643,192	July 1, 1997	Hirsh, et al.

20

FOREIGN PATENT DOCUMENTS

<u>PATENT NO.</u>	<u>ISSUE DATE</u>	<u>INVENTOR</u>
WO 94/00566	January 6, 1994	Cederholm-Williams, et al.
EU 0 592 242 A1	April 13, 1994	E.R. Squibb & Sons

- 25 The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

Disclosure of Invention

- 30 The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (e.g. six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field

(Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (i.e., less than 5 seconds) during only a very narrow four to five minute time period, totally impractical for the broad range of surgeries.

5 The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues
10 where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and it will provide fast clotting throughout even the longest surgeries. The thrombin
15 produced by this invention can be diluted in saline to provide precise, slower clotting times thereby allowing any preferred time from less than four seconds to longer than 2 minutes.

The procedure of the invention is comprised of three steps, the first two of which should occur at the same time:

20 1. Preparing a fraction enriched in prothrombin by use of Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Thrombinodulin and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.

25 2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

30 Industrial Applicability

The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

5 It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

10 It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time of less than thirty minutes.

15 It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol
20 fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

25 The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

The present invention further provides an efficient method of preparation.
30 Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological

components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

5 The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

10 These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

Brief Description of Drawings

Figure 1 is a perspective view of an apparatus for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins
15 therefrom.

Figure 2 is a sectional view of the thrombin processing unit where plasma is being admitted into a mixing syringe.

Figure 3 is a view similar to figure 2 in which the processing reagents are directed to the mixing syringe for processing the plasma into prothrombin and then
20 thrombin.

Figure 4 is a view similar to figures 2 and 3 where the thrombin is directed to a dispensing syringe after have first been filtered for particulate matter which could interfere with the thrombin being sprayed through a small orifice or expressed through a thin tube.

25 Figure 5 is a chart illustrating the effect of various ETOH concentrations in the final volume on the life span of fast clotting thrombin when the CaCl_2 is held constant at 0.023 μm .

Figure 6 is a chart illustrating the effect of various CaCl_2 concentrations in the final volume on the life span of fast clotting thrombin when the ETOH
30 concentration is held constant at 13.6%.

Figure 7 is a chart illustrating that the processing of the thrombin should occur in a glass syringe for a fast clotting preparation.

Figure 8 is a chart describing the contaminating proteins removed from the enriched thrombin fraction after mixture with ETOH, (13.6% in final volume) and CaCl_2 (.023 μm in final volume) and filtered for particulate matter.

5 Figure 9 is a view showing the life span of optimized thrombin preparation for fast clotting.

Figure 10 is a view showing the life span of optimized thrombin preparation diluted at 1:15 with sterile saline for slow clotting.

10 Figure 11 is a chart illustrating the conversion/activation period required for the enrichment of a prothrombin fraction and its conversion to stable thrombin by mixture with a precise solution of ETOH and CaCl_2 .

Figure 12 is a chart illustrating thrombin (Bovine) concentrations (activity) as it relates to speed of clotting.

Best Mode(s) for Carrying Out the Invention

15 Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figure 1.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

20 More particularly, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has a stop valve 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60.

25 Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. The plasma required for the thrombin processing unit is preferably 8 ml. so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and

adhesive proteins from the clotting and adhesive proteins processing unit 60. Referring to figure 2, a sealed bag 16 overlies the thrombin processing unit 40 to provide sterility until the thrombin storage syringe is introduced into a sterile surgical field. Prior to that, the thrombin processing unit is operated as shown in figure 2 within the sealed bag which is flexible and sized to preferably permit the movement of the syringes' plungers from the exterior of the bag. Fluid from the first branch 12 passes beyond a coupling 18 and into a manifold 22. The manifold 22 is equipped with a valve 24 that initially is directed to a mixing syringe 26 preferably formed from glass and capable of receiving a volume as great as 15 ml. The mixing syringe 26 includes a plunger 28, which when moved in the direction of the arrow A, draws the plasma from the passageway 12 and into the interior of the mixing syringe 26.

Referring to figure 3, the valve 24 is reoriented so that access can be gained between the mixing syringe 26 and the reagents found in ampoules 32, 34, each of which are operatively connected to the manifold 22 via a Y coupling 36 shown in figure 1. Access to the interior of either ampoule 32 or 34 can be had by squeezing the ampoule to rupture a frangible diaphragm. Alternatively, the intake 38 which receives the ampoule can be provided with a hollow spike which penetrates the diaphragm. In either event, the contents of both of the ampoules 32, 34 are received in the mixing syringe 26 by further retraction of the plunger 28 along the arrow A shown in figure 3. A first ampoule 32 is preferably provided with 2 ml. of ethanol providing an ETOH concentration in the final volume of 13.6% and the second ampoule 34 is preferably provided with 1 ml. calcium chloride providing a concentration in the final volume of .023µm. Alternatively, these reagents contained within the two ampoules 32, 34 can be premixed into a single ampoule and dispensed simultaneously. In one form of the invention, it is possible to introduce the ethanol first, then agitate the mixing syringe 26 and then follow with the calcium chloride, but the introduction of both simultaneously to the plasma are optimally combined, followed by brief agitation.

Once the ethanol and calcium chloride have been introduced into the mixing syringe 26, the valve 24 is reoriented so that the mixing syringe 26 is isolated. The contents are briefly agitated and allowed to incubate for about 20 minutes. Prior to pushing the contents out of the mixing syringe 26, the valve 24 is reoriented as

shown in figure 4 after which the plunger 28 is moved in the direction of the arrow B of figure 4. Because the valve 24 is now set to allow communication to the thrombin dispensing syringe 42, the contents within the mixing syringe 26 will be transferred from the mixing syringe 26 to the dispensing syringe 42. More specifically, the manifold 22 includes a recess within which a filter 44 is provided in the flow path between the mixing syringe 26 and the thrombin dispensing syringe 42. Particulate matter will be retained within the filter 44 prior to delivery of the thrombin to the dispensing syringe 42. Note that as fluid enters the dispensing syringe 42, the dispensing syringe plunger 46 moves in a direction opposite arrow B.

Referring back to figure 1, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 62 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 64 of the bag 62 for subsequent extraction by means of a clotting and adhesive protein dispensing syringe 66 contained in a sterile pouch 68. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive dispensing syringe 66, the two syringes can be decoupled from the processing set 10 and ganged together for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes should be stored at or below 4°C prior to usage.

Turning to figure 5, a graph is shown which illustrates how ethanol concentrations alter the life span of fast clotting thrombin where the calcium chloride content is held constant at point .023 μm . Note that at approximately 13.6% ethanol, its life span is shown to have been optimized and extend at least 240 minutes while its clotting time is substantially constant at under 5 seconds. The range between 8% and 18%, however, has utility.

Figure 6 varies the calcium chloride concentration in the thrombin while the ethanol is held constant at 13.6%. As shown, the thrombin life span where the calcium chloride concentration is at .023 μm of 250 mM calcium chloride appears optimized and extends to 360 minutes while maintaining a clot time under 5

seconds. The range between .011 μ m of 125mM and .045 μ m of 500mM, however, has utility.

Figure 7 reflects the differences in processing the thrombin where the thrombin mixing syringe 26 is formed from glass versus plastic. As can be shown, the speed of clotting is held to close to 5 seconds or less with a life span of 60 to 240 minutes in glass.

Figure 8 reflects the effect of using ethanol at 13.6% and calcium chloride at .023 μ m to reduce proteins which alter the clot time of the thrombin as compared to the original plasma. As can be seen in this graph, the major interfering proteins are so efficiently removed, that the clotting time of the thrombin is not only enhanced, but held substantially stable and constant.

Figure 9 shows in greater detail than that which is shown in figures 5 and 6 regarding the measured clot time as a function of life span for the optimized thrombin preparation, having been treated by 13.6% ethanol and .023 μ m calcium chloride. As shown, the life span extends to 360 minutes and the clot time varies from 3 to 4 seconds.

Figure 10 shows the effect of saline solution of the thrombin preparation optimized as in figure 9 with an ethanol concentration of 13.6% and a calcium chloride concentration of .023 μ m as a function of life span. When the thrombin has been diluted 1 to 1.5 with saline, the clot time has been extended from just above 20 seconds to just less than 30 seconds, and has a life span of up to 150 minutes.

Referring to figure 11, there shown is the benefit in allowing the thrombin contained in the mixing syringe 26 to reside therein after agitation for almost 20 minutes in order to assure the effectiveness of the filtration step in removing particulate matter for subsequent utilization. The time span for conversation and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer or expressing through a thin tube.

Figure 12 provides a prior art comparison of the activity of thrombin sourced from Bovine blood plasma as it relates to the speed of clotting, showing that

autologous thrombin derived from this invention provides a clotting speed equivalent to 100 iu/ml of Bovine thrombin.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without
5 departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

CLAIMS

We Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

- 5 obtaining a blood product from the patient;
 sequestering plasma from the product;
 enriching the prothrombin in a plasma fraction;
 converting the prothrombin to thrombin, and
 filtering particulate from the thrombin.

10 Claim 2 - The method of claim 1 further including the step of altering the clotting time.

 Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

15 Claim 4 - The method of claim 3 wherein the converting step includes adding CaCl_2 .

 Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining plasma.

 Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with saline.

20 Claim 7 - The method of claim 6 including filtering the plasma by weight size and protein binding.

 Claim 8 - A method for producing autologous thrombin which is stable for more than 15 minutes, the steps including:

25 sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

 Claim 9 - Autologous thrombin which provides fast clotting for more than 15 minutes.

 Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

30 Plasma;
 Ethanol (ETOH);
 CaCl_2 .

Claim 11 - The composition of claim 10 wherein ETOH is present at 13.6% and CaCl_2 is present at .023 μm both by volume.

Claim 12 - A method for preparing thrombin comprising:

obtaining plasma;

5 adding ETOH and CaCl_2 to the plasma, forming a composition;

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

10 Claim 13 - A manifold with an integrated filter for preparing thrombin from plasma, comprising:

a dock for a plasma syringe on the manifold;

a receptacle for a solution of CaCl_2 and ETOH and a dock on the manifold therefore;

15 a dock for a thrombin receiving syringe to receive the thrombin through the filter;

the integrated filter located on the manifold and just upstream the thrombin receiving syringe.

Claim 14 - A fluid receiving system, comprising, in combination;

20 a thrombin processing set and an adhesive protein processing set operatively coupled thereto, in fluid communication therewith.

Claim 15 - The system of claim 14 wherein said fluid receiving system includes an inlet for receiving blood product therein,

and first and second blood product receiving branches communicating with said inlet for distributing the blood product to each said processing set.

25 Claim 16 - The system of claim 15 wherein said blood product from said first branch leads to a manifold in fluid communication with syringe means and reagent means via valve means, and said thrombin processing unit is ensconced in a sealed bag.

30 Claim 17 - The system of claim 16 wherein said syringe means includes a mixing syringe and a thrombin dispensing syringe.

Claim 18 - The system of claim 17 wherein said reagent means includes a first ampoule and a second ampoule.

Claim 19 - The system of claim 18 wherein said first ampoule contains ethanol.

Claim 20 - The system of claim 19 wherein said second ampoule contains a source of calcium ions.

5 Claim 21 - The system of claim 17 wherein said reagent means includes a mixture of ethanol and calcium ions.

Claim 22 - The system of claim 20 wherein said manifold includes a filter interposed in a flow path between said mixing syringe and said thrombin dispensing syringe.

10 Claim 23 - The system of claim 22 wherein said blood product from said second branch leads to said adhesive protein processing set including an interior chamber having a nose portion which communicates with an adhesive dispensing syringe.

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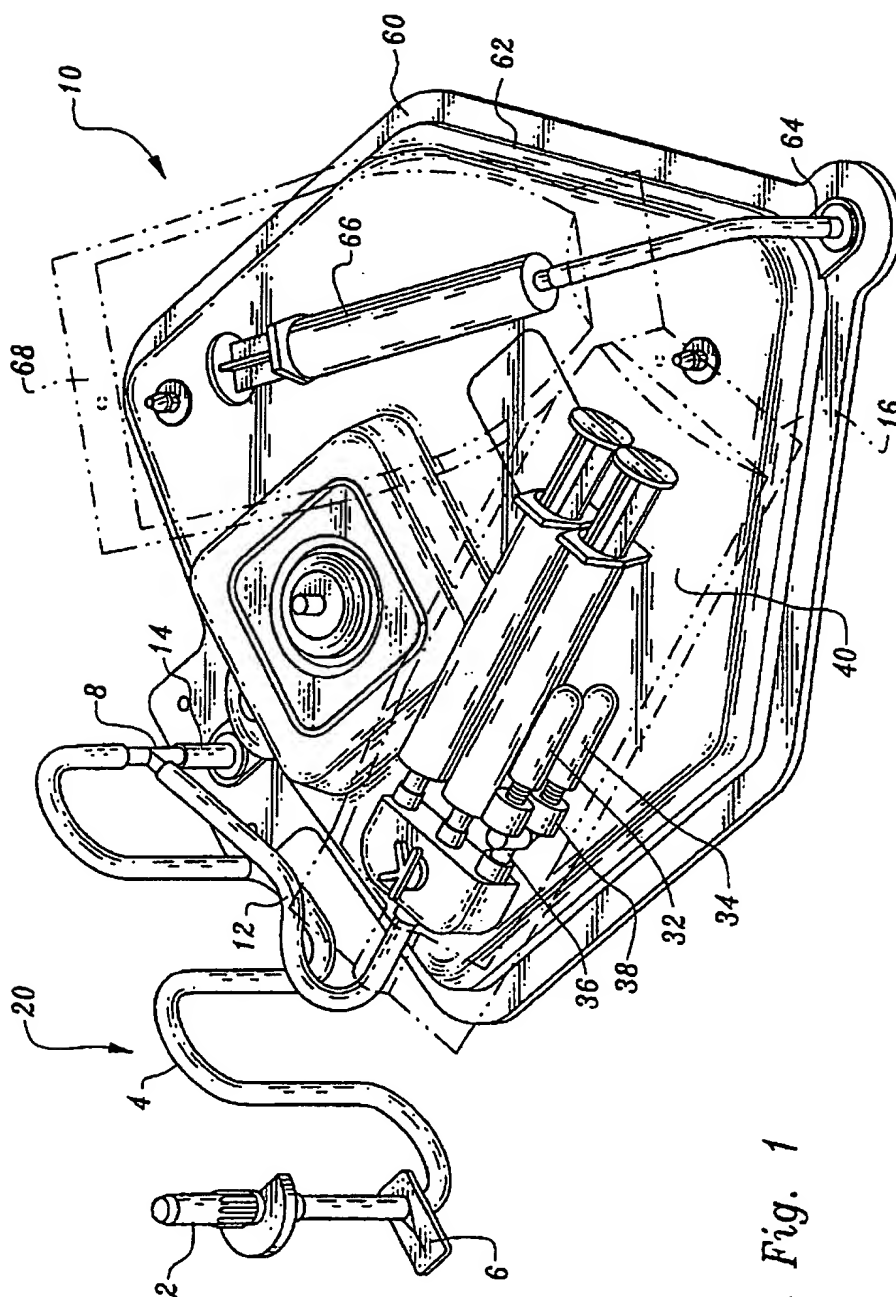


Fig. 1

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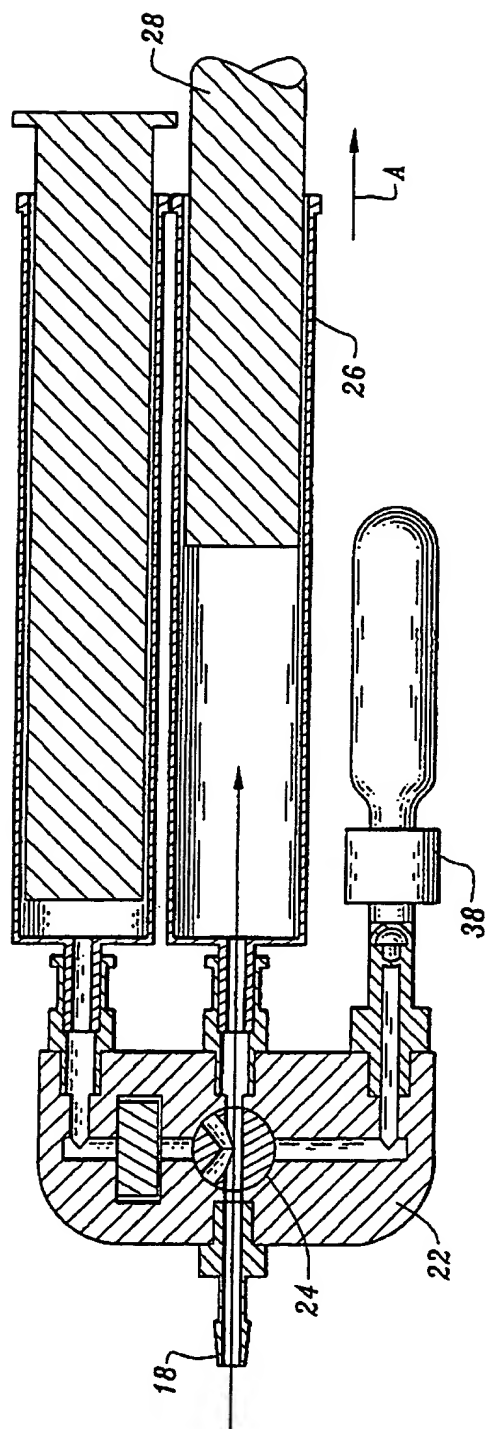


Fig. 2

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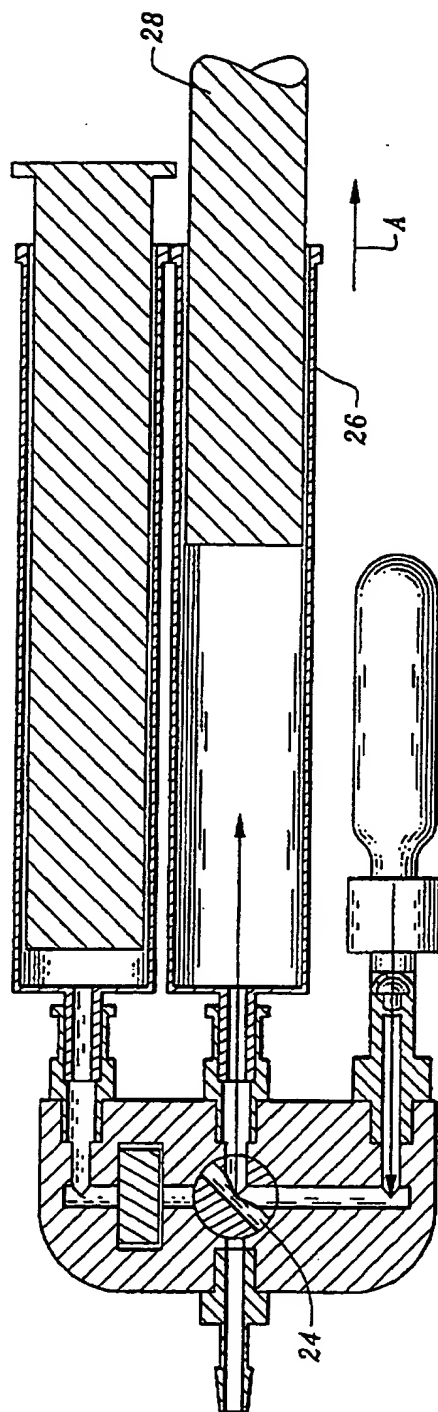


Fig. 3

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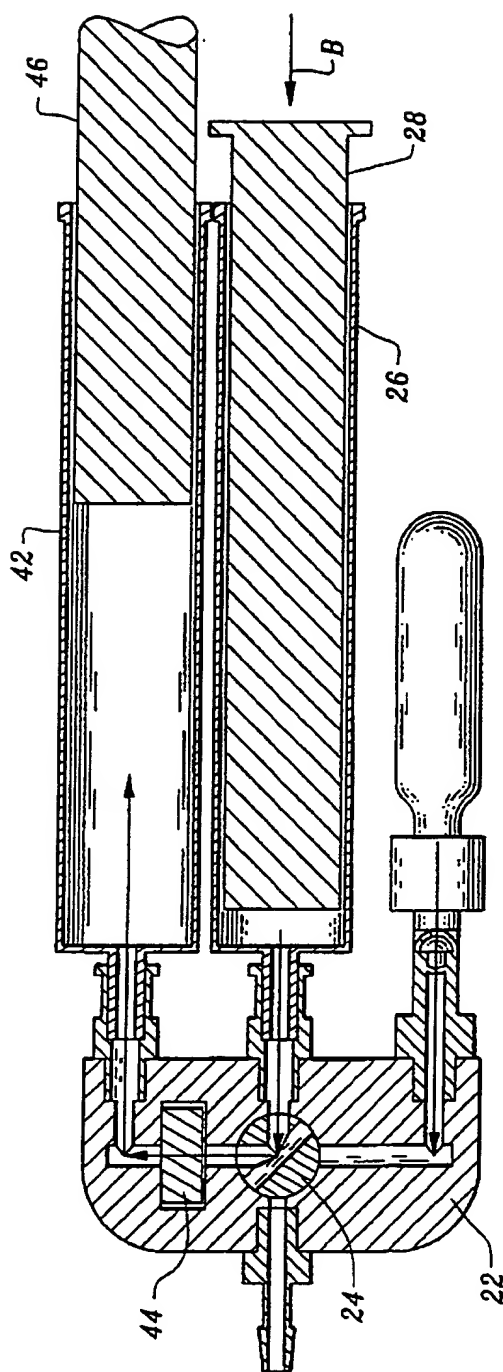
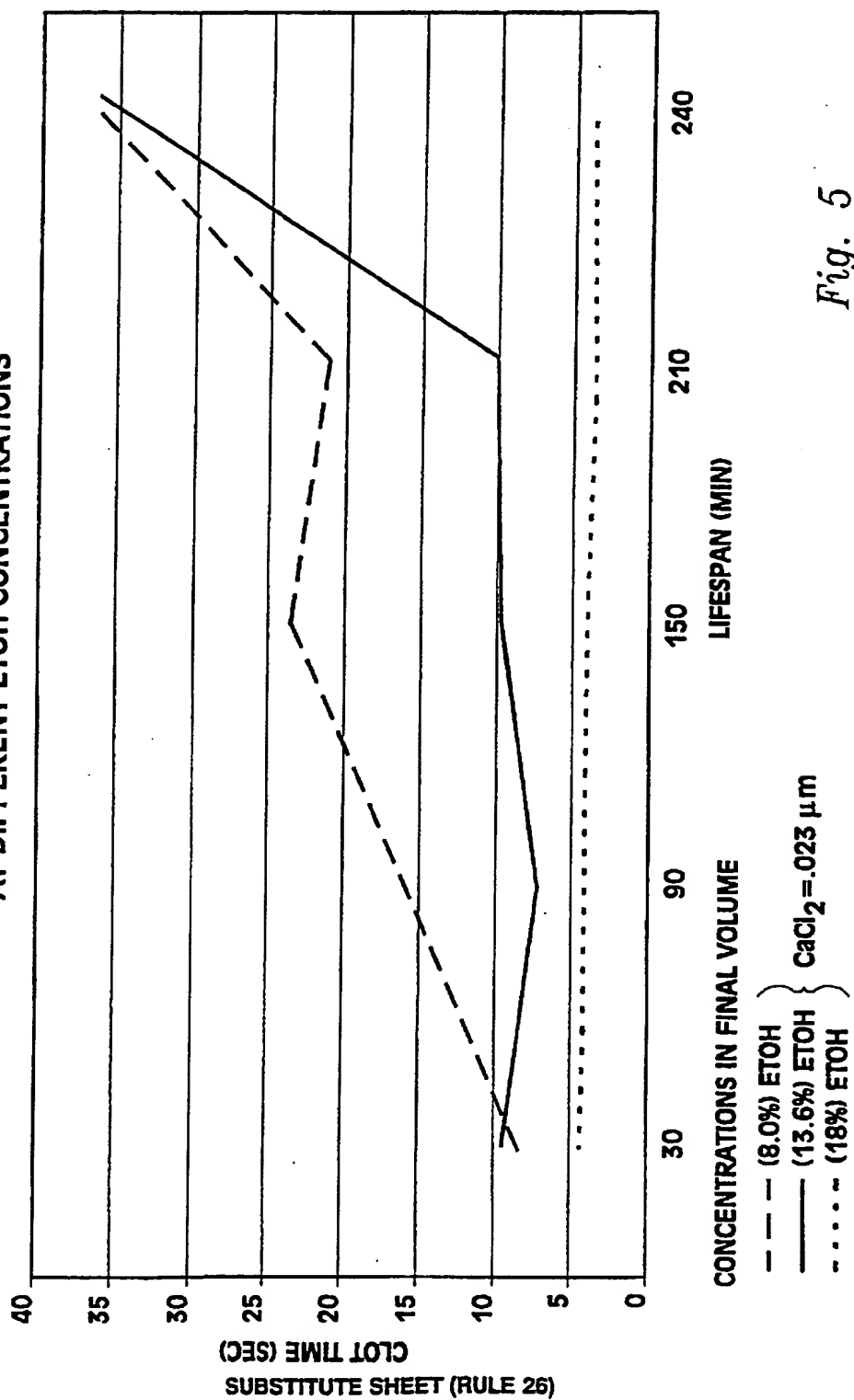


Fig. 4

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LIFESPAN OF FAST CLOTTING THROMBIN FRACTIONATED
AT DIFFERENT ETOH CONCENTRATIONS

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LIFESPAN OF FAST CLOTTING THROMBIN AT DIFFERENT CaCl₂ CONCENTRATIONS

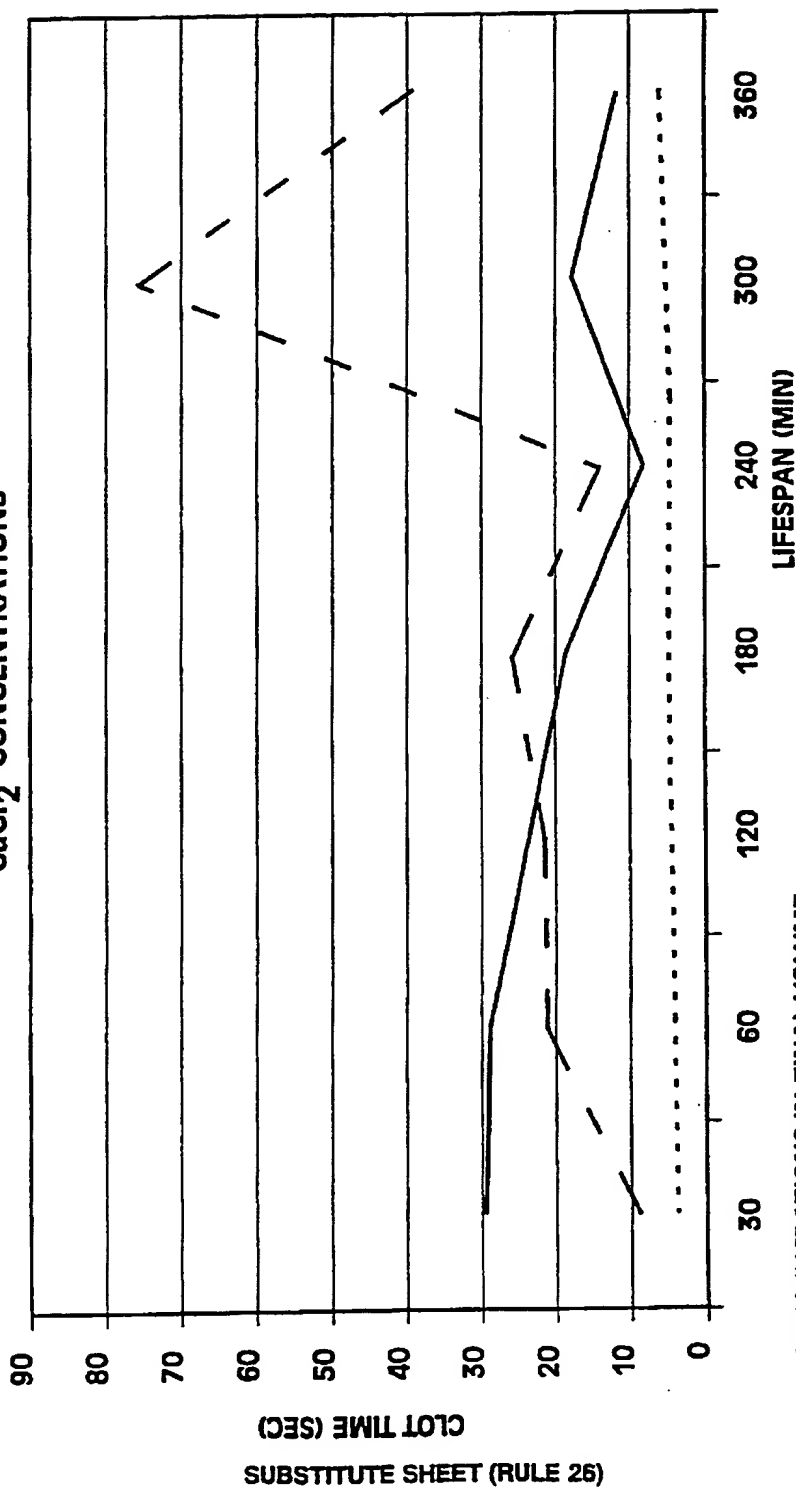


Fig. 6

CONCENTRATIONS IN FINAL VOLUME

- (.011 μm) OF 125mM CaCl₂
 - (.023 μm) OF 250mM CaCl₂
 - - - - (.045 μm) OF 500mM CaCl₂
- 13.6% ETOH

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LIFESPAN AND CLOTTING SPEED OF THROMBIN PROCESSED IN CONTAINERS OF DIFFERENT MATERIALS

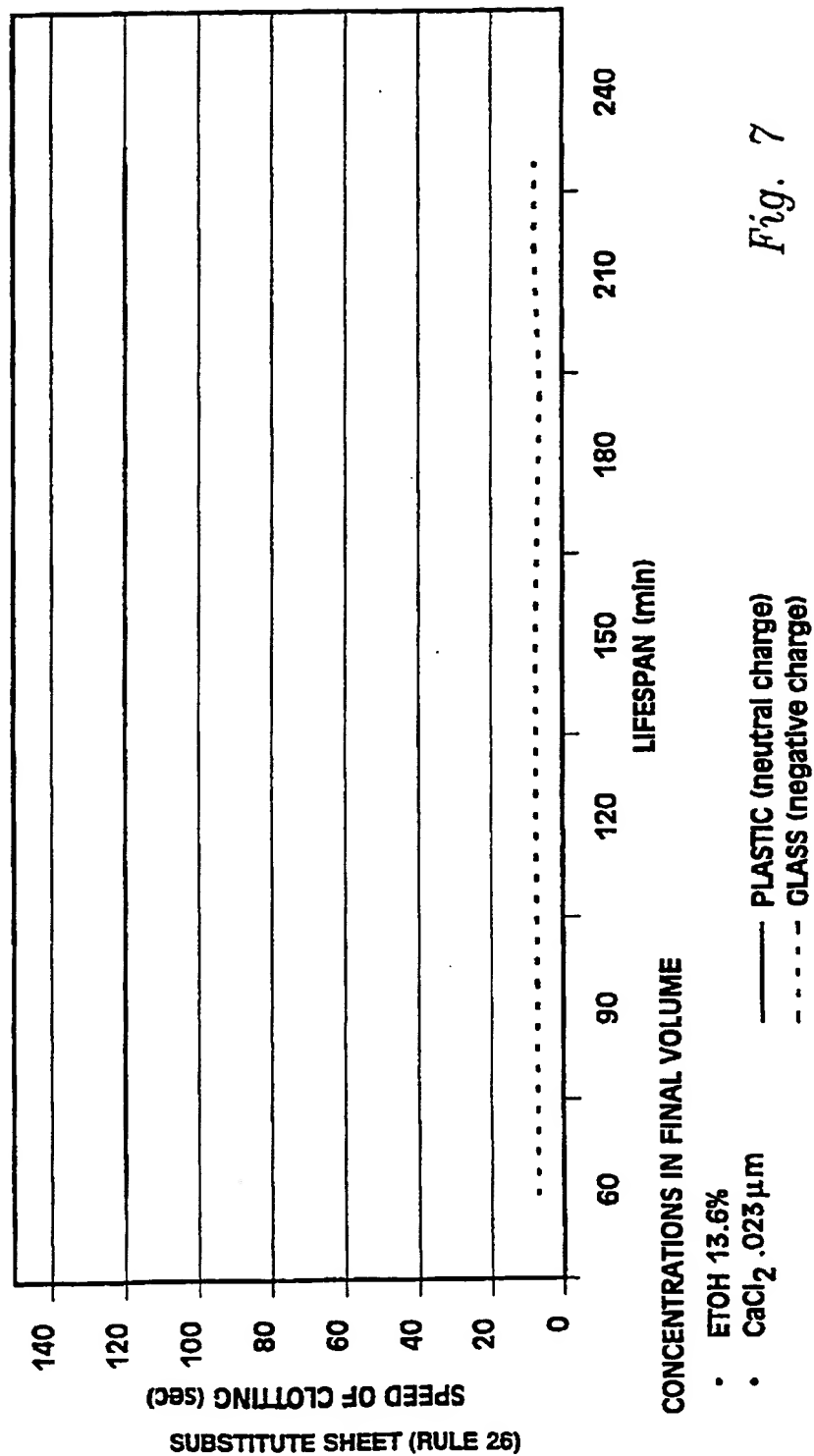
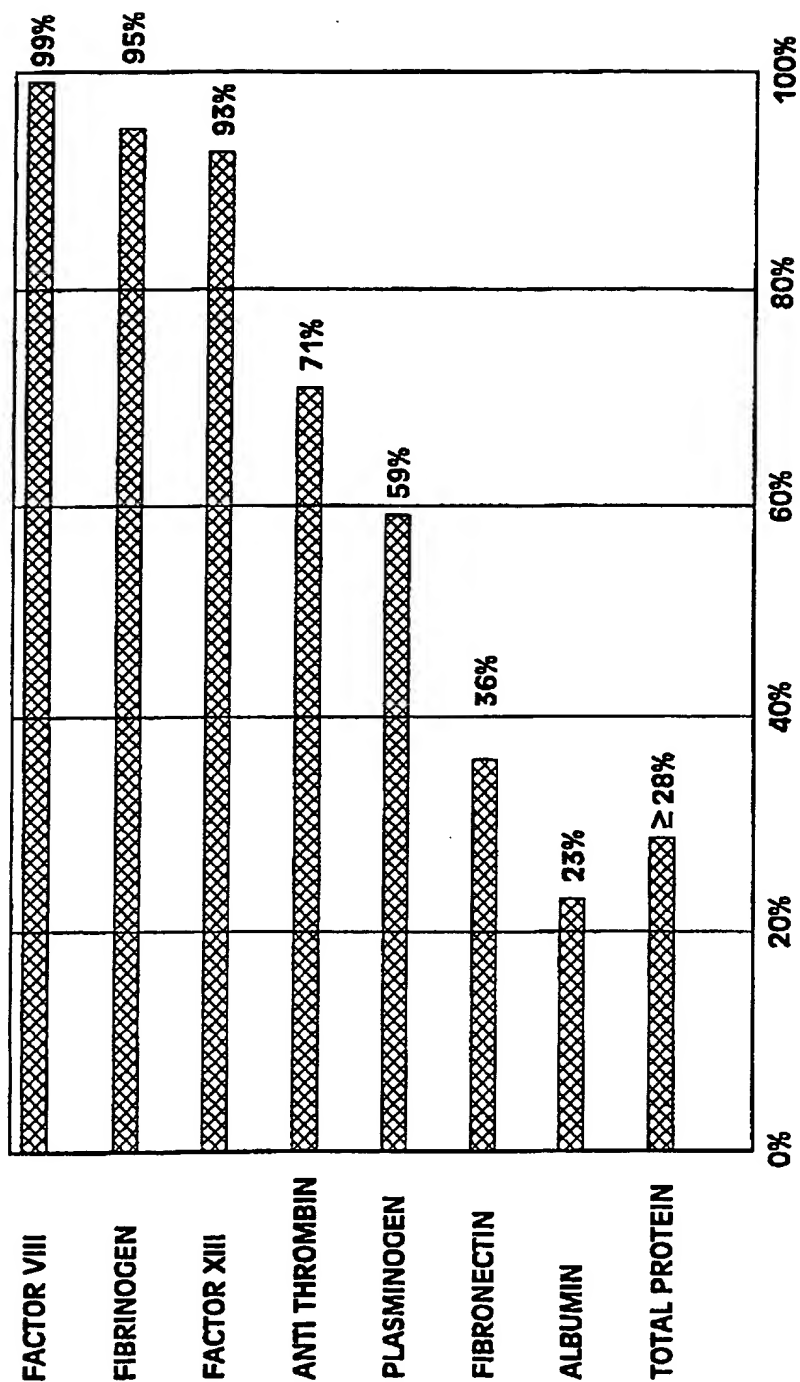


Fig. 7

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REDUCTION IN CONTAMINATING PROTEINS FROM OPTIMIZED
ENRICHED THROMBIN FRACTION AS COMPARED TO THE ORIGINAL PLASMA



CONCENTRATIONS IN FINAL VOLUME

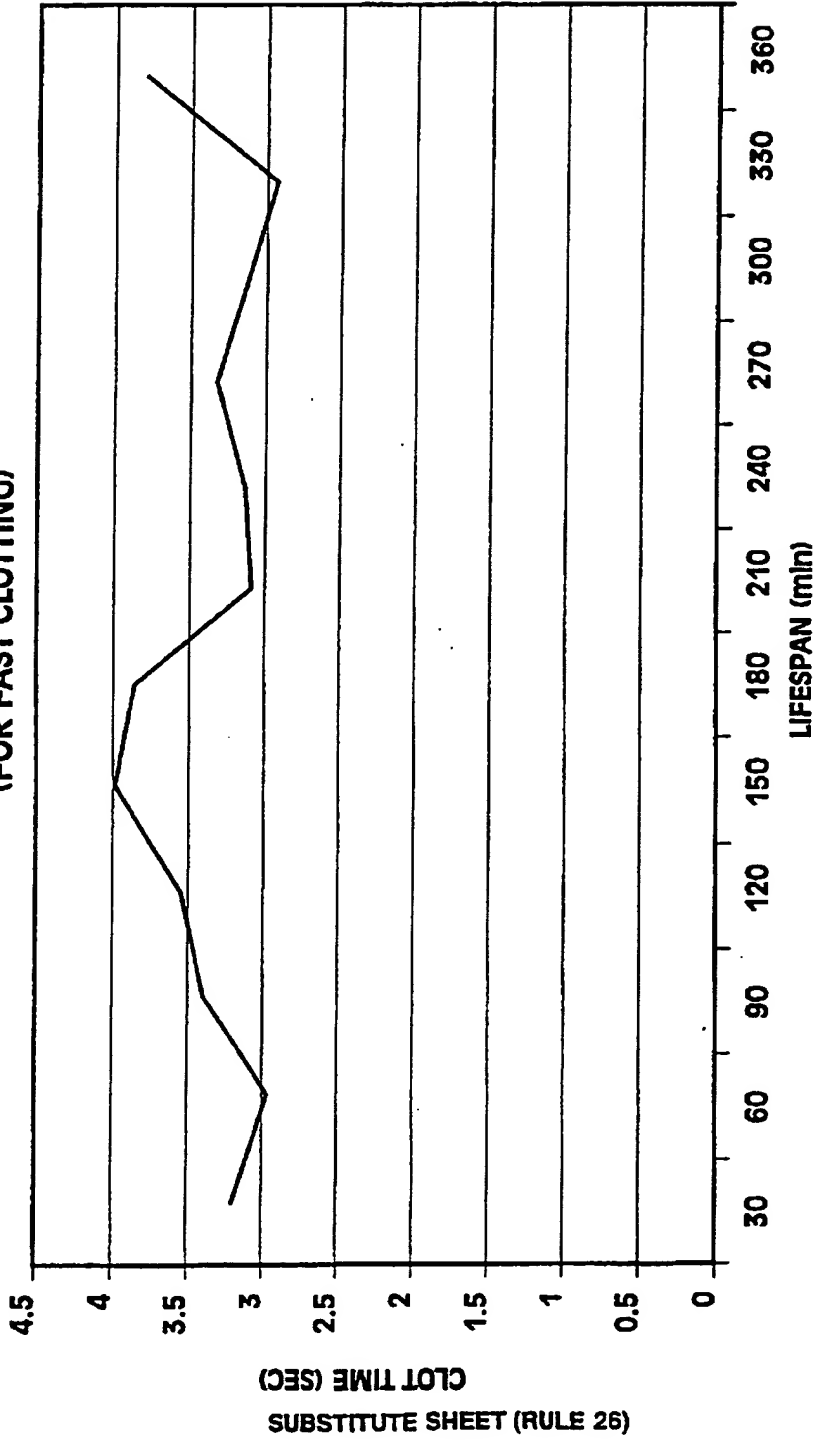
• ETOH 13.6%

• CaCl₂ .023μm

Fig. 8

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LIFESPAN OF OPTIMIZED THROMBIN PREPARATION (FOR FAST CLOTTING)



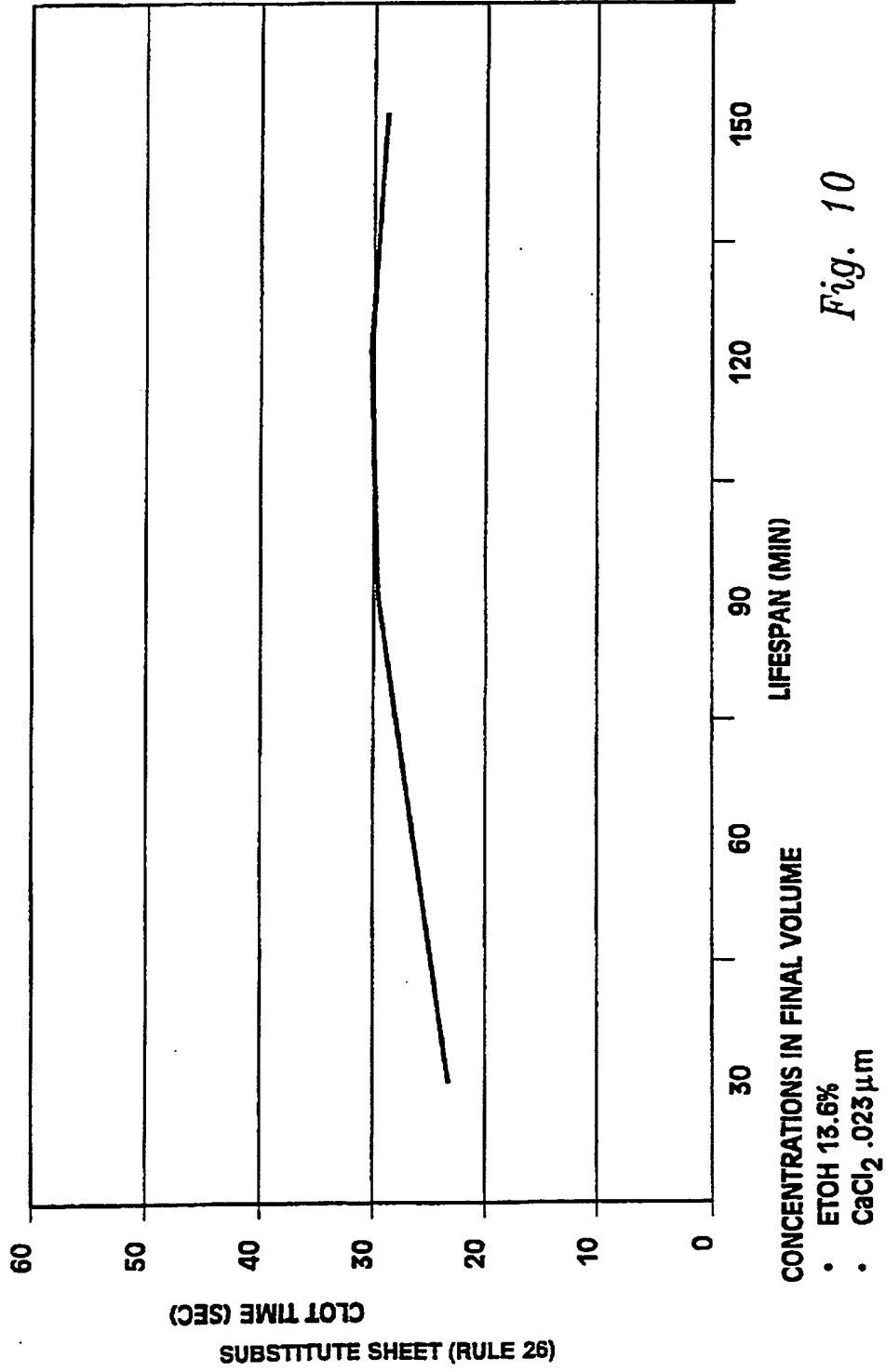
CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl₂ .023 μm

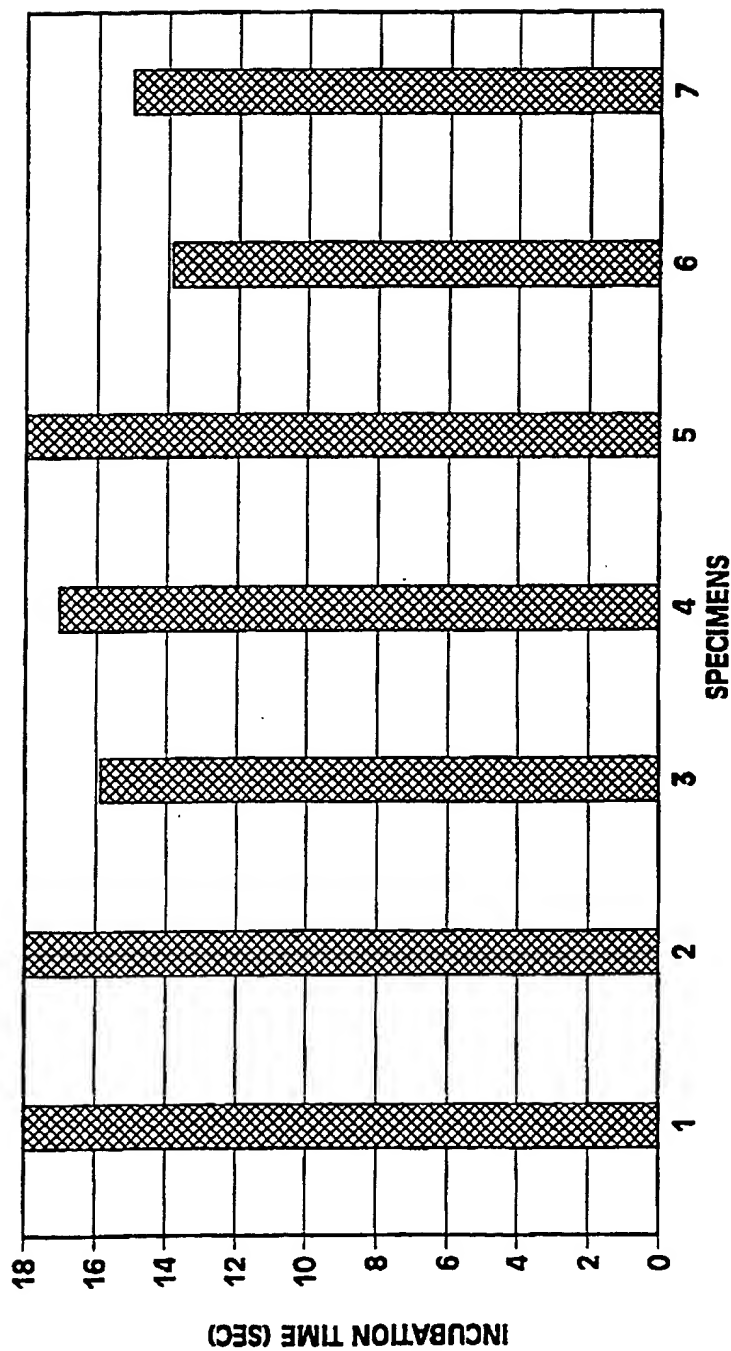
Fig. 9

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**LIFESPAN OF THROMBIN PREPARATION
(DILUTED 1:1.5 FOR SLOW CLOTTING)**



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TYPICAL CONVERSION/ACTIVATION PERIODS TO CONVERT OPTIMIZED, ENRICHED
PROTHROMBIN FRACTION TO THROMBIN

SUBSTITUTE SHEET (RULE 26)

CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl₂ 0.023 μm

Fig. 11

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BOVINE THROMBIN
CONCENTRATION VS. CLOTTING TIME

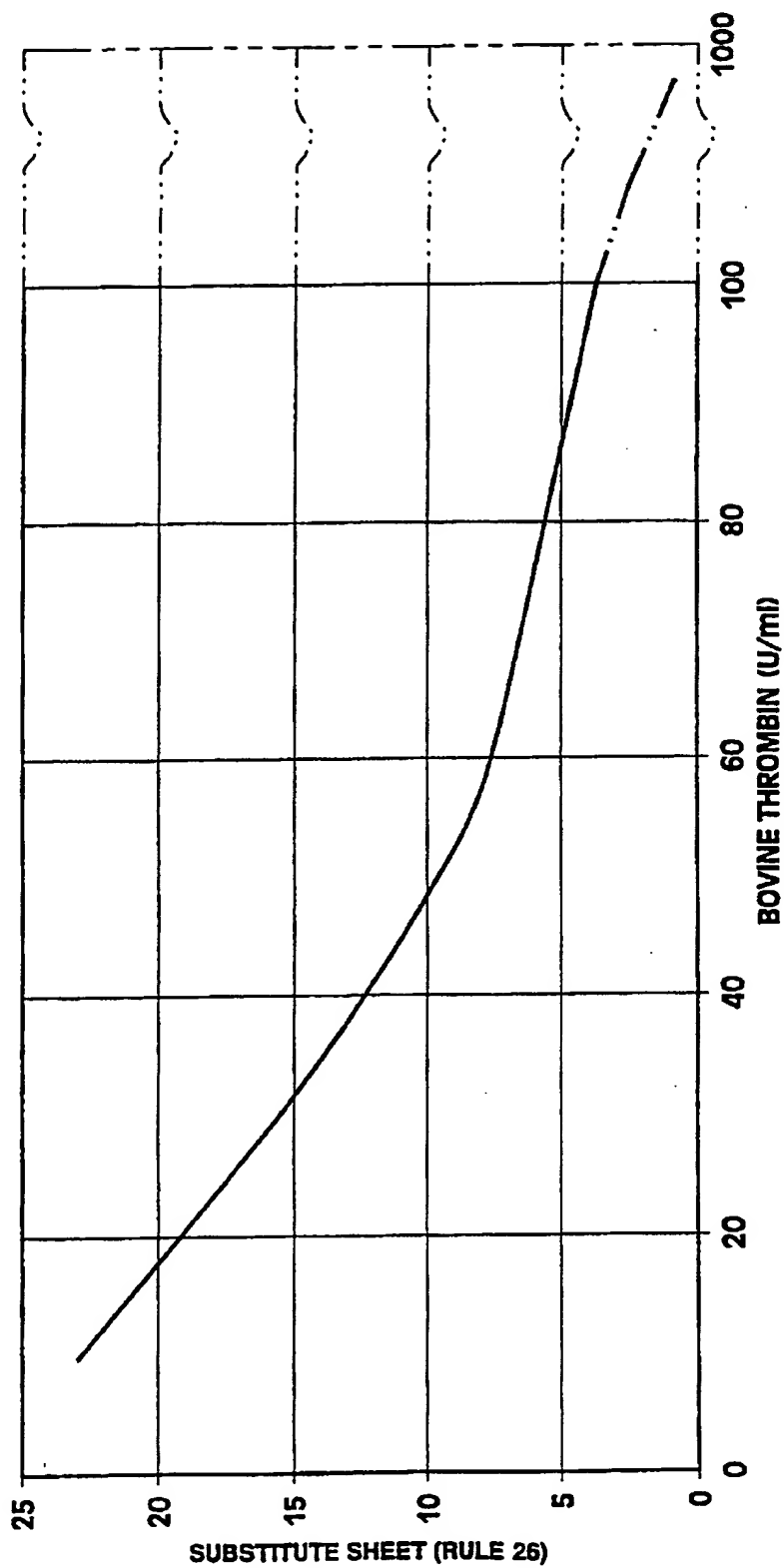


Fig. 12

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/US99/16698

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61M 37/00; C12N 5/00; C12Q 1/02; G01N 33/566

US CL :Please See Extra Sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94/05566 A1 (E.R. SQUIBB & SONS, INC.) 06 January 1994. See entire reference.	1-12
Y	US 5,232,024 A (WILLIAMS) 03 August 1993. See entire reference.	13-17
Y	US 5,460,945 A (SPRINGER et al.) 24 October 1995. See entire reference, in particular cols. 15-21.	13-23
Y	US 5,037,390 A (RAINES et al.) 06 August 1991. See entire reference.	13, 16-18 20-23
Y	US 5,643,192 A (HIRSH et al.) 01 July 1997. See cols. 1-4	1-12

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 NOVEMBER 1999

Date of mailing of the international search report

13 JAN 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16698

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,575,779 A (BARRY) 19 November 1996. See cols. 1-3.	14-17
Y	US 5,510,102 A (COCHRUM) 23 April 1996. See cols. 1-4.	1-12
Y	SZCZEPANSKI et al. Thrombin Clotting Time and Fibrin Polymerization in Liver Cirrhosis. Materia Medica Polona. 1994, Vol. 26, No. 3, pages 87-90. See entire reference.	4, 6, 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/16698

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/185, 435/252.33, 514/184, 435/37, 424/94.64, 514/2, 435/7.24, 435/69.2, 514/21, 435/17, 536/21, 530/350, 514/56, 604/246, 137/883

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

514/185, 435/252.33, 514/184, 435/37, 424/94.64, 514/2, 435/7.24, 435/69.2, 514/21, 435/17, 536/21, 530/350, 514/56, 604/246, 137/883